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Molecular Characterization of Multidrug-Resistant Strains of *Salmonella enterica* Serotype Typhimurium and Monophasic Variant (*S.* 4,[5],12:i:–) Isolated from Human Infections in Italy

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Abstract

Salmonella enterica serovar Typhimurium (STM) represents the prevalent cause of foodborne gastroenteritis in Italy with the majority of isolates exhibiting multidrug resistance. A resistant pattern that includes ampicillin (A), streptomycin (S), sulfonamide (Su), and tetracycline (T) (ASSuT) but lacks resistance to chloramphenicol (C) has recently emerged in Italy among strains of STM and of its monophasic variant, *S. enterica* subspecies enterica serovar *S.* 4,[5],12:i:–. With the aim to evaluate their clonal relationships, 553 strains of STM and *S.* 4,[5],12:i:– with the ASSuT and ACSSuT resistance patterns isolated in Italy from human infections between 2003 and 2006 were characterized by pulsed-field gel electrophoresis (PFGE) according to the PulseNet-Europe protocol and nomenclature. Among both the STM and *S.* 4,[5],12:i:– ASSuT strains, the predominant PFGE profile was STYMXB.0079 (53.2–73.0% of strains, respectively), while the STM ACSSuT strains belonged to the STYMXB.0061 (37.2% of strains) and STYMXB.0067 (29.9% of strains). Bionumerics cluster analysis of the nonunique PFGE profiles showed that more than 90% of ASSuT and ACSSuT-resistant strains were included in two distinct clusters with a genetic homology of 73% each other, suggesting that the ASSuT-resistant strains belong to a same clonal lineage different from that of the ACSSuT strains. Phage typing showed that 23% of the ASSuT STM strains were not typeable and 22.3% were U302. The same phage types were observed among the ASSuT strains of *S.* 4,[5],12:i:–. A different figure was observed for the ACSSuT strains: the STM isolates mostly belonged to DT104 (70.2%), while none of the *S.* 4,[5],12:i:– strains belonged to this phage type. This study indicates that the tetra-resistant ASSuT strains of STM and *S.* 4,[5],12:i:–, increasingly isolated in Italy, belong to a same clonal lineage and that the *S.* 4,[5],12:i:– strains circulating in our country mainly derive from this STM clonal lineage.

Introduction

Salmonella enterica serovar Typhimurium (STM) is a common cause of nontyphoidal salmonellosis worldwide and represents the second most prevalent serovar isolated in Europe, after *Salmonella* Enteritidis (European Food Safety Authority, 2007; http://www.ecdc.europa.eu/pdf/ECDC_epi_report_2007.pdf). In Italy, since 2001, STM represents the prevalent cause of human infections (Graziani *et al.*, 2008) and in 2006 it accounted for the 44.2% of all human *Salmonella* isolates (Galetta *et al.*, 2008).

The public health impact of STM infections is further increased by the high rate of antimicrobial resistance associated with this serovar (Rabatsky-Ehr *et al.*, 2004). In particular, an epidemic multidrug-resistant (MDR) clonal lineage of STM, namely, DT104 phage type (Weill *et al.*, 2006), has emerged in Europe and the United States since the 1980s. STM DT104 is characterized by a penta-resistant pattern including resistance to ampicillin (A), chloramphenicol (C), streptomycin (S), sulfonamide (Su), and tetracycline (T) (ACSSuT).

Recently, strains of STM with a tetra-resistant pattern ASSuT, with or without additional resistances, but lacking resistance to chloramphenicol, have emerged in Italy (Busani *et al.*, 2004). While the frequency of the ACSSuT pattern among the Italian human isolates of STM remained constant over the years, the frequency of the tetra-resistant pattern

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increased from 7% in the period 1999–2001 to 34.1% in 2006, when the penta-resistant strains accounted for 29.6% (Busani *et al.*, 2004; Graziani *et al.*, 2008).

In Italy the tetra-resistant pattern is also prevalent (Barone *et al.*, 2008; Galetta *et al.*, 2008) among human isolates belonging to *S. enterica* subspecies serovar 4,[5],12:i:– (*S.* 4,[5],12:i:–), defined as a monophasic variant of *Salmonella* Typhimurium. This serovar shares almost all antigenic factors with STM, but it lacks the second-phase flagellar antigen encoded by the *fljB* gene (Echeita *et al.*, 2001; Zamperini *et al.*, 2007).

The monophasic serovar has been associated with human infections in some European countries since 1997 (Echeita *et al.*, 1999; Mossong *et al.*, 2007; Meakins *et al.*, 2008) and represents the third most frequent serovar from human infections in Italy since 2004 (Galetta *et al.*, 2006, 2007, 2008).

STM and monophasic strains with the ASSuT pattern have been frequently isolated from animal sources—in particular, swine and product thereof (http://www.izsvenezie.it/dnn/Portals/0/salmonellosi/EnterVet%202006.pdf; Barone *et al.*, 2008; Romani *et al.*, 2008). Both human and animal strains usually did not belong to DT104 (Busani *et al.*, 2004; Graziani *et al.*, 2008) and often did not react with any of the phages included in the panel currently used for phage typing (Anderson *et al.*, 1977). Owing to this feature, molecular typing appears to be the best tool for subtyping these isolates, to investigate their clonal relationships, to track their spread, and to better understand their epidemiology. Among the molecular typing techniques, pulsed-field gel electrophoresis (PFGE) has became the reference method all over the world (Swaminathan *et al.*, 2001; http://www.cdc.gov/pulsenet/; http://www.pulsenet-europe.org/); it is considered the gold standard for molecular subtyping of *Salmonella* and has been shown to be highly effective in epidemiological studies conducted on different serovars of *S. enterica* (Harbottle *et al.*, 2006; Herrero *et al.*, 2006; Michael *et al.*, 2006).

This study was undertaken to characterize STM and *S.* 4,[5],12:i:– strains with the ASSuT pattern isolated from human infections in Italy during the period 2003–2006, with the aim of evaluating their clonal origin and their relationships with the ACSSuT strains of STM and *S.* 4,[5],12:i:– isolated in the same period.

Materials and Methods

Bacterial strains

During the period from January 2003 to December 2006, 732 STM and 191 *S.* 4,[5],12:i:– epidemiologically unrelated strains were received at the Istituto Superiore di Sanità in the framework of the National surveillance system for enteric infections (http://www.iss.it/ente). Serotyping of *Salmonella* spp. isolates was performed by slide agglutination according to Kauffmann-White scheme (Grimont and Weill, 2007). Strains were definitively assigned to serovar Typhimurium or *S.* 4,[5],12:i:– on the basis of the presence or the absence of the *fljB* gene tested by PCR (Echeita *et al.*, 2002).

Antimicrobial susceptibility testing

The strains, many of which described in previous studies (Graziani *et al.*, 2008), were tested for antimicrobial susceptibility by the disk diffusion method (Clinical and Laboratory Standards Institute, 2006). The antimicrobials tested included the following antibiotic disks (Becton Dickinson) and concentrations (mg): nalidixic acid (Nx) 30, ampicillin (A) 10, cefotaxime (Ctx) 30, ciprofloxacin (Cp) 5, chloramphenicol (C) 30, gentamicin (G) 10, kanamycin (K) 30, streptomycin (S) 10, sulfonamides (Su) 300, tetracycline (T) 30, and trimethoprim–sulfamethoxazole (SXT) 1.25/23.75, according to the Enter-net reference panel (Threlfall *et al.*, 2003). *Escherichia coli* ATCC 25922 was used as control strain in each experiment. Isolates showing tetra-resistant (ASSuT) and penta-resistant (ACSSuT) profiles, with or without additional resistances to other classes of antimicrobials, were included in this study.

PFGE

Genetic relatedness was determined by PFGE analysis after digestion of DNA with *Xba*I restriction enzyme (New England Biolabs, Ipswich, MA) and according to Salmgene and PulseNet standardized protocol (Peters *et al.*, 2003; Ribot *et al.*, 2006). *S. enterica* serovar Braenderup H9812 was used as the molecular size marker (Hunter *et al.*, 2005). Dendrogram and cluster analysis were performed using algorithms available within the BioNumerics software package (v. 4.61; Applied Maths, Sint-Martens-Latem, Belgium). The percent similarity between different chromosomal fingerprints was scored by the Dice coefficient. The unweighted pair group method with arithmetic means, with a 1.00% tolerance limit and 1.00% optimization, was used to obtained the dendrogram. DNA profiles differing by one or more DNA fragments were considered as distinct patterns. Strains with a coefficient of similarity $\geq$80% were considered as closely related genetically.

The locally analyzed profiles of the strains were uploaded to the international database established at the Health Protection Agency (HPA; Colindale, London, United Kingdom), compared with the profiles in the international database and named, as agreed with the PulseNet Europe, with a six-letter code followed by a four-digit numerical identifier, for example, STYMXB.0006 (Lukinmaa *et al.*, 2006; http://www.cdc.gov/pulsenet/index).

Phage typing

It was possible to carry out phage typing on a subset of strains: 357 STM and 69 *S.* 4,[5],12:i:, randomly selected among each PFGE profile. The strains were phage typed according to the protocol reported by Anderson *et al.* (1977) using phage dilutions and the interpretative guidelines kindly supplied by the HPA, Colindale. Strains that reacted to phages but did not conform to any defined pattern were classified as reacts but does not conform, and those that did not react with any of the typing phages were considered unable to be typed (DTNT).

Results

The main antimicrobial resistance patterns of the 732 STM and 191 *S.* 4,[5],12:i:– strains collected through the surveillance system between 2003 and 2006 are reported in Table 1.

The 553 STM and *S.* 4,[5],12:i:– strains showing the ASSuT or ACSSuT patterns, with or without additional resistances, were further characterized by PFGE and partly by phage typing.

All of the isolates were typeable using PFGE. The results of PFGE profile analysis for each of clusters containing more than one isolate are shown in Fig. 1.

Table 1. Antimicrobial Resistance Pattern of *Salmonella* Typhimurium and *S.* 4,[5],12:i:– Strains

R-type	Salmonella *Typhimurium* n *(%)*	S. *4,[5],12:i:–* n *(%)*
ASSuT ± other	203 (27.7%)	137 (71.7%)
ACSSuT ± other	204 (27.9%)	9 (4.7%)
1–3 resistances	161 (22%)	27 (14.1%)
4 or + resistances	42 (5.7%)	11 (5.7%)
Susceptible	122 (16.7%)	7 (3.7%)
Total	732	191

ASSuT: ampicillin (A), streptomycin (S), sulfonamide (Su), and tetracycline (T); ACSSuT: ampicillin (A), chloramphenicol (C), streptomycin (S), sulfonamide (Su), and tetracycline (T).

All the profiles obtained are summarized in Table 2 according to the resistance pattern. The 203 ASSuT STM strains were categorized into 54 different PFGE profiles, 44 of which corresponded to a single isolate and are indicated as "other" in Table 2. The predominant profile was STYMXB.0079, which accounted for 53.2% of the isolates, followed by STYMXB.0339 (9.8%) and STYMXB.0010 (4.4%).

The 137 ASSuT *S.* 4,[5],12:i:– isolates belonged to 17 different profiles, 13 of which corresponded to a single isolate. The STYMXB.0079 profile was prevalent also in this group of strains (73%), followed as well by STYMXB.0010 (9.5%) and STYMXB.0339 (5.8%).

The coefficients of similarity (*F*) of the predominant STYMXB.0079 with the STYMXB.0339 and STYMXB.0010 profiles were 0.84 and 0.91, respectively (Fig. 1).

The STYMXB.0079 and STYMXB.0010 profiles were also observed among the nine ACSSuT *S.* 4,[5],12:i:– isolates examined. Conversely, the 204 penta-resistant STM isolates mostly (67.1%) belonged to the STYMXB.0061 and STYMXB.0067 profiles, which showed an *F*-value of 0.95. We also observed 41 PFGE profiles among ACSSuT strains, 31 of which were unique.

Overall, two large clusters with a genetic homology of 73% were identified. Cluster A included 275 out of the 283 (97.2%) ASSuT strains, while cluster B, included 169 out of the 179 (94.4%) ACSSuT isolates, regardless of STM or *S.* 4,[5],12:i:– serovar. A close genetic relationship ($F > 0.80$) was observed among the strains belonging to each cluster. The few exceptions included eight tetra-resistant strains with PFGE profiles (STYMXB.0086, STYMXB.0051, and STYMXB.0058) grouping in cluster B, and 10 penta-resistant strains with profiles grouping in cluster A (STYMXB.0079, STYMXB.0010, and STYMXB.0080).

Phage typing was performed for 357 STM and 69 *S.* 4,[5],12:i:–, including tetra-resistant and penta-resistant strains. The ASSuT strains mainly belonged to DTNT or U302: 23% and 22.3% of STM strains, and 46% and 27.9% of *S.* 4,[5],12:i:– strains, respectively. Conversely, most of the ACSSuT STM strains (70.2%) belonged to DT104, as expected, while none of the ACSSuT *S.* 4,[5],12:i:– belonged to this phage type.

The associations between PFGE profiles and phage types are described in detail in Tables 3 and 4.

Discussion

Surveillance of *Salmonella* infections in Italy showed that, since 2001, STM has become the most common serovar associated with human infections, reaching 44.2% of the cases in 2006 (Graziani *et al.*, 2005; Galetta *et al.*, 2008). During the same period, an increase in infections associated with *S.* 4,[5],12:i:– has also been observed, with this serovar accounting for 5.4%

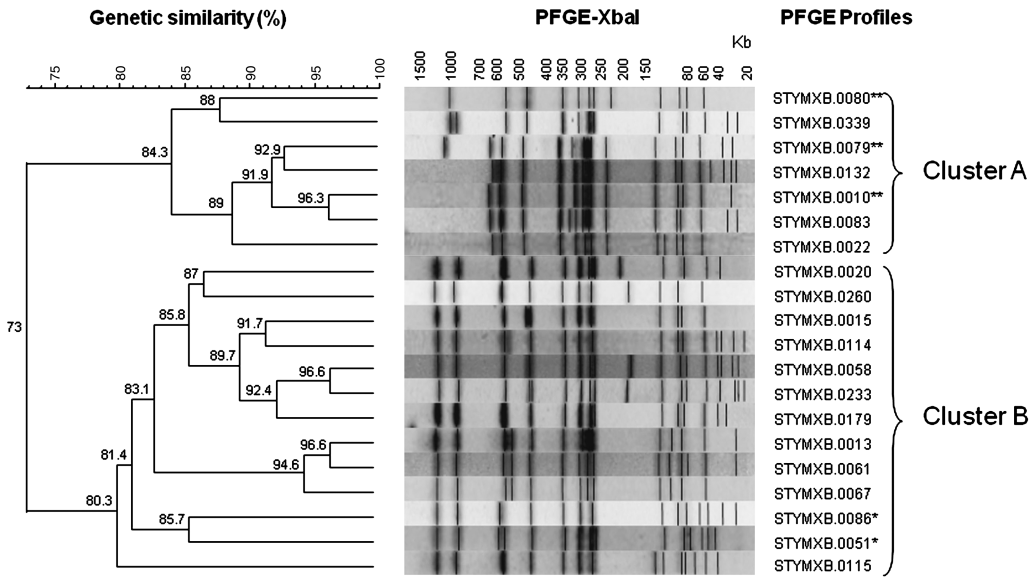

FIG. 1. Dendrogram generated by BioNumerics software showing the results of cluster analysis of 20, nonunique, pulsed-field gel electrophoresis (PFGE) patterns described among tetra-resistant and penta-resistant *Salmonella* Typhimurium and *S.* 4,[5],12:i:– strains. Similarity analysis was performed using the Dice coefficient (Opt: 1.00%; Tol: 1.00%), and clustering was by unweighted pair group method with arithmetic means. Cluster A indicates the tetra-resistant isolates, and cluster B the penta-resistant isolates. *PFGE profiles of tetra-resistant isolates grouping in cluster B. **PFGE profiles showed by both the R-types.

Table 2. Distribution of PFGE Profiles Among Tetra-R and Penta-R *Salmonella* Typhimurium and *S.* 4,[5],12:i:– Strains

	Salmonella *Typhimurium*		S. *4,[5],12:i:–*	
PFGE profile (cluster[a])	*ASSuT ± other*	*ACSSuT ± other*	*ASSuT ± other*	*ACSSuT ± other*
STMXB.0079 (A)	108 (53.2)	5 (2.5)	100 (73)	3 (33.3)
STMXB.0339 (A)	20 (9.8)	—	8 (5.8)	—
STMXB.0010 (A)	9 (4.4)	—	13 (9.5)	1 (11.1)
STMXB.0132 (A)	5 (2.5)	—	—	—
STMXB.0022 (A)	4 (2)	—	—	—
STMXB.0083 (A)	4 (2)	—	—	—
STMXB.0080 (A)	1 (0.5)	—	3 (2.2)	1 (11.1)
STMXB.0061 (B)	—	76 (37.2)	—	1 (11.1)
STMXB.0067 (B)	—	61 (29.9)	—	—
STMXB.0114 (B)	—	6 (2.9)	—	—
STMXB.0179 (B)	—	5 (2.4)	—	—
STMXB.0020 (B)	—	4 (2.0)	—	—
STMXB.0015 (B)	—	4 (2.0)	—	—
STMXB.0260 (B)	—	3 (1.5)	—	—
STMXB.0013 (B)	—	2 (1.0)	—	—
STMXB.0115 (B)	—	2 (1.0)	—	—
STMXB.0233 (B)	—	2 (1.0)	—	—
STMXB.0058 (B)	3 (1.5)	3 (1.5)	—	—
STMXB.0086 (B)	3 (1.5)	—	—	—
STMXB.0051 (B)	2 (0.9)	—	—	—
Other profiles	44 (21.7)	31 (15.1)	13 (9.5)	3 (33.3)
Total	203 (100.0)	204 (100.0)	137 (100.0)	9 (100.0)

[a]Clusters A and B were defined by Bionumeric cluster analysis as shown in Fig. 1.
PFGE, pulsed-field gel electrophoresis.

of human salmonellosis in 2006 and ranking third after STM and *S.* Enteritidis (Galetta *et al.*, 2008).

As in other countries (Rabatsky-Ehr *et al.*, 2004; Weill *et al.*, 2006; Meakins *et al.*, 2008), the strains of STM and *S.* 4,[5], 12:i:– isolated in Italy are frequently MDR (Barone *et al.*, 2008; Graziani *et al.*, 2008; this study). However, while the DT104-associated ACSSuT pattern has been prevalently reported in other countries, the STM strains isolated in Italy presented either the ACSSuT pattern or an emerging ASSuT pattern (Graziani *et al.*, 2008). This pattern was also largely prevalent among the *S.* 4,[5],12:i:– isolates that only rarely showed the ACSSuT pattern (Barone *et al.*, 2008; this study).

In the present study, PFGE typing of a representative number of STM and *S.* 4,[5],12:i:– ASSuT strains isolated in Italy during the last 4 years showed a strong association between this resistance pattern and particular PFGE profiles. In particular, 67.5% of the ASSuT strains of STM belonged to the prevalent PFGE profile STYMXB.0079 (53.2%) and, to a lesser

Table 3. Association Between PFGE Profile and Phage Types of the 357 *Salmonella* Typhimurium Strains According to Resistance Pattern

	PFGE profile							
	ASSuT ± other				*ACSSuT ± other*			
Phage type	*STMXB.0079*	*STMXB.0339*	*STMXB.0010*	*Other*	*STMXB.0061*	*STMXB.0067*	*STMXB.0079*	*Other*
DTNT	27	3	—	9	2	—	2	5
U302	27	1	1	9	4	4	2	5
DT7	15	—	—	6	—	—	—	—
RDNC	3	2	—	9	—	—	—	7
DT120	7	—	6	9	2	2	—	11
DT20	3	—	—	—	—	—	—	—
DT194	2	—	—	2	—	—	—	—
DT104	—	—	—	4	54	45	—	32
DT193	—	—	—	8	—	—	—	—
DT22	—	7	—	—	—	—	—	—
Other	1	3	—	6	—	—	—	10
Total	85	16	7	62	62	51	4	70

DTNT, unable to be typed; RDNC, reacts does not conform.

Table 4. Association Between PFGE Patterns and Phage Types of the 69 Strains of *S.* 4,[5],12:i:– According to Resistance Pattern

	PFGE profile					
	ASSuT ± other		*ACSSuT ± other*			
Phage type	*STMXB.0079*	*Other*	*STMXB.0010*	*STMXB.0079*	*STMXB.0080*	*Other*
U302	16	1	—	1	—	3
DTNT	22	6	1	1	1	—
DT7	1	1	—	—	—	—
RDNC	4	—	—	—	—	—
DT120	1	5	—	—	—	—
Other	1	3	—	—	—	1
Total	45	16	1	2	1	4

DTNT, unable to be typed; RDNC, reacts does not conform.

extent, to the two closely related profiles, STYMXB.0010 (4.4%) and STYMXB.0339 (9.8%). Similar percentages were observed for *S.* 4,[5],12:i:– serovar; 88.3% of ASSuT strains belonged to STYMXB.0079 (73%), STYMXB.0010 (9.5%), and STYMXB.0339 (5.8%). These PFGE profiles were also observed among the ACSSuT *S.* 4,[5],12:i:– strains, but not among the STM penta-resistant isolates, 67.1% of which belonged to STYMXB.0061 (37.2%) and STYMXB.0067 (29.9%). In general, the cluster analysis also showed that more than 90% of the penta-resistant and tetra-resistant strains were included in two distinct clusters, suggesting that the ASSuT strains belong to a same clonal lineage, different from that of the ACSSuT strains.

A few of the STM and *S.* 4,[5],12:i:– strains with the STYMXB.0079 profile showed the penta-resistant pattern, but none of them belonged to DT104. Moreover, some of these strains were negative for the presence of *Salmonella* Genomic Island 1 when tested in a previous study by Amar *et al.* (2008). These findings suggest that they could derive from the ASSuT clonal lineage by the acquisition of resistance to chloramphenicol. On the contrary, the eight tetra-resistant strains grouping in cluster B could harbor a defective *Salmonella* Genomic Island 1 that may have lost the gene encoding for chloramphenicol-florphenicol resistance (Boyd *et al.*, 2002; Carattoli *et al.*, 2002).

The emergence and spread of the ASSuT clonal lineage, herein described, deserves consideration. The isolation of ASSuT STM strains has been reported in other countries, but with lower prevalences and different strain characteristics. In Denmark, ASSuT strains represented 10.2% of all the STM isolated in 2003, and an ASSuT STM strain belonging to phage type U302 was responsible for a prolonged outbreak in the same year (Ethelberg *et al.*, 2004). In France, a 3.8% prevalence of ASSuT has been reported among STM strains isolated from 1993 to 2003, with different combinations of phage types and PFGE types (Weill *et al.*, 2006).

Differently from the Italian isolates, the ASSuT pattern has been frequently associated with phage type DT193 in several studies. In the United Kingdom, an ASSuT strain of STM DT193 was responsible for an extensive outbreak in 1989; in the following years the ASSuT pattern was detected in 38% of STM DT193 isolates from human infections (Hampton *et al.*, 1995). The ASSuT pattern was reported in 14.2% of the STM isolated in Spain between 2001 and 2003, with 40.5% of strains being DT193 (Soler *et al.*, 2006). In the United States, the ASSuT pattern with additional resistance to kanamycin was found to be common among STM isolated from swine during 1997–2000 (Gebreyes and Altier, 2002). Again, most of the isolates belonged to phage type DT193 and showed a high genetic homology. In the United States, the tetra-resistant pattern with additional resistance to kanamycin was also observed in 9% of the human strains isolated during 1997–1998 (Rabatsky-Ehr *et al.*, 2004). In that study, 36% of the isolates were DTNT but, as stated by the authors, DT193 could not be excluded, as the typing panel used did not include the additional phages. In the present study, only 8 out of the 170 tetra-resistant STM strains examined belonged to phage type DT193, and none of them belonged to STYMXB.0079 or closely related PFGE profiles. As far as *S.* 4,[5],12:i:– is concerned, several studies have reported that MDR isolates mainly present the ACSSuT pattern and belonged to the DT104 complex and clonal lineage (Echeita *et al.*, 2001; de la Torre *et al.*, 2003; Amavisit *et al.*, 2005). However, a strain of *S.* 4,[5],12:i:– with the ASSuT pattern associated with an outbreak in Luxembourg belonged to phage type DT193 and showed an STYMXB.0031 PFGE profile, according to Pulsenet Europe nomenclature (Mossong *et al.*, 2007).

Conclusions

This study indicates that the tetra-resistant ASSuT strains of STM and *S.* 4,[5],12:i:–, increasingly isolated in Italy, belong to a same clonal lineage and that the *S.* 4,[5],12:i:– strains circulating in our country mainly derive from this STM clonal lineage.

The results of this study show that *S.* 4,[5],12:i:– strains isolated in Italy are mainly ASSuT and that the rare ACSSuT strains do not belong to the DT104 clonal lineage.

Further studies are needed to characterize the resistance genes present in the tetra-resistant ASSuT isolates and their location. Aimed surveillance efforts will also be needed to better understand the epidemiology of this MDR clone and its potential for international spreading.

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Disclosure Statement

No competing financial interests exist.

References

Amar CF, Arnold C, Bankier A, Dear PH, Guerra B, Hopkins KL, Liebana E, Mevius DJ, and Threlfall EJ. Real-time PCRs and fingerprinting assays for the detection and characterization of *Salmonella* Genomic Island-1 encoding multidrug resistance: application to 445 European isolates of *Salmonella*, *Escherichia coli*, Shigella, and Proteus. Microb Drug Resist 2008;14:79–92.

Amavisit P, Boonyawiwat W, and Bangtrakulnont A. Characterization of *Salmonella enterica* serovar Typhimurium and monophasic *Salmonella* serovar 1,4,[5],12:i:- isolates in Thailand. J Clin Microbiol 2005;43:2736–2740.

Anderson ES, Ward LR, Saxe MJ, and de Sa JD. Bacteriophage-typing designations of *Salmonella* Typhimurium. J Hyg 1977;78: 297–300.

Barone L, Dal VA, Pellissier N, Vigano A, Romani C, and Pontello M. Emergence of *Salmonella* Typhimurium monophasic serovar: determinants of antimicrobial resistance in porcine and human strains. Ann Ig 2008;20:199–209.

Boyd D, Cloeckaert A, Chaslus-Dancla E, and Mulvey MR. Characterization of variant *Salmonella* genomic island 1 multidrug resistance regions from serovars Typhimurium DT104 and Agona. Antimicrob Agents Chemother 2002;46:1714–1722.

Busani L, Graziani C, Battisti A, Franco A, Ricci A, Vio D, Digiannatale E, Paterlini F, D'Incau M, Owczarek S, Caprioli A, and Luzzi I. Antibiotic resistance in *Salmonella enterica* serotypes Typhimurium, Enteritidis and Infantis from human infections, foodstuffs and farm animals in Italy. Epidemiol Infect 2004;132:245–251.

Carattoli A, Filetici E, Villa L, Dionisi AM, Ricci A, and Luzzi I. Antibiotic resistance genes and *Salmonella* genomic island 1 in *Salmonella enterica* serovar Typhimurium isolated in Italy. Antimicrob Agents Chemother 2002;46:2821–2828.

Clinical and Laboratory Standards Institute. 2006. Institute Performance Standards for Antimicrobial Susceptibility Testing; Sixteenth Informational Supplement. CLSI/NCCLS document M100-S16, Wayne, PA.

de la Torre E, Zapata D, Tello M, Mejia W, Frias N, Garcia Pena FJ, Mateu EM, and Torre E. Several *Salmonella enterica* subsp. enterica serotype 4,5,12:i:- phage types isolated from swine samples originate from serotype typhimurium DT U302. J Clin Microbiol 2003;41:2395–2400.

Echeita MA, Aladueña A, Cruchaga S, and Usera MA. Emergence and spread of an atypical *Salmonella enterica* subsp. enterica serotype 4,5,12:i:- strain in Spain. J Clin Microbiol 1999;37:3425.

Echeita MA, Herrera S, and Usera MA. Atypical, fljB-Negative *Salmonella enterica* subsp. enterica strain of serovar 4,5,12:i:2 appears to be a monophasic variant of serovar Typhimurium. J Clin Microbiol 2001;39:2981–2983.

Echeita MA, Herrera S, Garaizar J, and Usera MA. Multiplex PCR-based detection and identification of the most common *Salmonella* second-phase flagellar antigens. Res Microbiol 2002;153:107–113.

Ethelberg S, Lisby M, Torpdahl M, Sorensen G, Neimann J, Rasmussen P, Bang S, Stamer U, Hansson HB, Nygard K, Baggesen DL, Nielsen EM, Molbak K, and Helms M. Prolonged restaurant-associated outbreak of multidrug-resistant *Salmonella* Typhimurium among patients from several European countries. Clin Microbiol Infect 2004;10:904–910.

European Food Safety Authority. 2007. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2006. The EFSA Journal (130). Available at: www.efsa.europa.eu.

Galetta P, Filetici E, Dionisi AM, Benedetti I, Arena S, Owczarek S, Lana S, Bella A, Scavia G, Minelli F, Marziano M, Grazian iC, Caprioli A, Luzzi I, and Laboratori della rete Enter-Net Italia. Enter-net: sorveglianza delle infezioni da patogeni enterici. Isolamenti di *Salmonella* spp., *E. coli* verotossigenici e *Campylobacter* spp. da infezioni umane in Italia nel 2004. Notiziario dell'Istituto Superiore di Sanità 2006;19:11–16. (In Italian.)

Galetta P, Dionisi AM, Filetici E, Benedetti I, Arena S, Owczarek S, Lana S, Bella A, Scavia G, Rizzo C, Seyler T, Minelli F, Marziano ML, Graziani C, Ciofi degli Atti M, Caprioli A, Luzzi I, and Laboratori della rete Enter-net Italia. Enter-net: sorveglianza delle infezioni da patogeni enterici. Isolamenti di *Salmonella* spp., *E. coli* produttori di verocitotossina e *Campylobacter* spp. da infezioni umane e da fonti ambientali in Italia nel 2005. Notiziario Istituto Superiore di Sanità 2007;20:11–17. (In Italian.)

Galetta P, Dionisi AM, Filetici E, Benedetti I, Arena S, Owczarek S, Lana S, Bella A, Scavia G, Rizzo C, Seyler T, Minelli F, Marziano ML, Graziani C, Ciofi degli Atti M, Caprioli A, Luzzi I, and Laboratori della rete Enter-net Italia. Enter-net: sorveglianza delle infezioni da patogeni enterici. Isolamenti di *Salmonella* spp., *E. coli* produttori di verocitotossina e *Campylobacter* spp. da infezioni umane e da fonti ambientali in Italia nel 2006. Notiziario Istituto Superiore di Sanità 2008;21:11–17. (In Italian.)

Gebreyes WA and Altier C. Molecular characterization of multidrug-resistant *Salmonella enterica* subsp. enterica serovar Typhimurium isolates from swine. J Clin Microbiol 2002;40: 2813–2822.

Graziani C, Galetta P, Busani L, Dionisi AM, Filetici E, Ricci A, Caprioli A, and Luzzi I. 2005. Infezioni da *Salmonella*: diagnostica, epidemiologia e sorveglianza., vol. 5. Rapporti ISTISAN. (In Italian.)

Graziani C, Busani L, Dionisi AM, Lucarelli C, Owczarek S, Ricci A, Mancin M, Caprioli A, and Luzzi I. Antimicrobial resistance in *Salmonella enterica* serovar Typhimurium from human and animal sources in Italy. Vet Microbiol 2008;128: 414–418.

Grimont PAD and Weill FX. *Antigenic Formulae Fort the* Salmonella *Serovars*, 9th edition. Paris, France: WHO Collaborating Centre for Reference and Research on *Salmonella*, 2007.

Hampton MD, Threlfall EJ, Frost JA, Ward LR, and Rowe B. *Salmonella* typhimurium DT 193: differentiation of an epidemic phage type by antibiogram, plasmid profile, plasmid fingerprint and *Salmonella* plasmid virulence (spv) gene probe. J Appl Bacteriol 1995;78:402–408.

Harbottle H, White D, McDermott P, Walker R, and Zhao S. Comparison of multilocus sequence typing, pulsed-field gel electrophoresis, and antimicrobial susceptibility typing for characterization of *Salmonella enterica* serotype Newport isolates. J Clin Microbiol 2006;44:2449–2457.

Herrero A, Rodicio M, González-Hevia M, and Mendoza M. Molecular epidemiology of emergent multidrug-resistant *Salmonella enterica* serotype Typhimurium strains carrying the virulence resistance plasmid pUO-StVR2. J Antimicrob Chemother 2006;57:39–45.

Hunter SB, Vauterin P, Lambert-Fair MA, Van Duyne MS, Kubota K, Graves L, Wrigley D, Barrett T, and Ribot E. Establishment of a universal size standard strain for use with the PulseNet standardized pulsed-field gel electrophoresis protocols: converting the national databases to the new size standard. J Clin Microbiol 2005;43:1045–1050.

Lukinmaa S, Nakari UM, Liimatainen A, and Siitonen A. Genomic diversity within phage types of *Salmonella enterica* ssp. enterica serotypes Enteritidis and Typhimurium. Foodborne Pathog Dis 2006;3:97–105.

Meakins S, Fisher IS, Berghold C, Gerner-Smidt P, Tschäpe H, Cormican M, Luzzi I, Schneider F, Wannett W, Coia J, Echeita A, Threlfall EJ, and Enter-net participants. Antimicrobial drug resistance in human nontyphoidal *Salmonella* isolates in Europe 2000–2004: a report from the Enter-net International Surveillance Network. Microb Drug Resist 2008;14:31–35.

Michael GB, Cardoso M, and Schwarz S. Molecular analysis of *Salmonella enterica* subsp. enterica serovar Agona isolated from slaughter pigs. Vet Microbiol 2006;112:43–52.

Mossong J, Marques P, Ragimbeau C, Huberty-Krau P, Losch S, Meyer G, Moris G, Strottner C, Rabsch W, and Schneider F. Outbreaks of monophasic *Salmonella enterica* serovar 4,[5],12:i:- in Luxembourg, 2006. Eur Surveill 2007;12:E11–E12.

Peters TM, Maguire C, Threlfall EJ, Fisher IS, Gill N, and Gatto AJ. The Salm-gene project-a European collaboration for DNA fingerprinting. Eur Surveill 2003;8:46–50.

Rabatsky-Ehr T, Whichard J, Rossiter S, Holland B, Stamey K, Headrick M, Barrett T, Angulo F, and NARMS Working Group. Multidrug-resistant strains of *Salmonella enterica* Typhimurium, United States, 1997–1998. Emerg Infect Dis 2004; 10:795–801.

Ribot EM, Fair MA, and Gautom R. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and Shigella for PulseNet. Foodborne Pathog Dis 2006;3:59–67.

Romani C, Aleo A, Pellissier N, Vigano A, and Pontello M. Characterization of multi-drug resistance in *Salmonella* strains isolated from animals. Ann Ist Super Sanita 2008;44:292–300.

Soler P, Gonzalez-Sanz R, Bleda MJ, Hernandez G, Echeita A, and Usera MA. Antimicrobial resistance in non-typhoidal *Salmonella* from human sources, Spain, 2001–2003. J Antimicrob Chemother 2006;58:310–314.

Swaminathan B, Barrett TJ, Hunter SB, and Tauxe RV. PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance, United States. Emerg Infect Dis 2001;7:382–389.

Threlfall E, Fisher I, Berghold C, Gerner-Smidt P, Tschäpe H, Cormican M, Luzzi I, Schnieder F, Wannet W, Machado J, and Edwards G. Antimicrobial drug resistance in isolates of *Salmonella enterica* from cases of salmonellosis in humans in Europe in 2000: results of international multi-centre surveillance. Eur Surveill 2003;8:41–45.

Weill FX, Guesnier F, Guibert V, Timinouni M, Demartin M, Polomack L, and Grimont PAD. Multidrug resistance in *Salmonella enterica* serotype Typhimurium from humans in France (1993 to 2003). J Clin Microbiol 2006;44:700–708.

Zamperini K, Soni V, Waltman D, Sanchez S, Theriault EC, Bray J, and Maurer JJ. Molecular characterization reveals *Salmonella enterica* serovar 4,[5],12:i:– from poultry is a variant Typhimurium serovar. Avian Dis 2007;51:958–964.

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